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T H E S I S.

ANAPHYLAXIS IN MAN AND GUINEA-PIGS.

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ANAPHYLAXIS.

This phenomenon is also known as Hypersensibility or Supersensitiveness. It is a condition of exaggerated sensibility of the organism to foreign substances.

When the blood serum of one animal is injected into an animal of different species, the injected animal in many cases is apparently not influenced by the injection. But if the experiment be repeated after a certain interval, the injected animal usually shews signs of marked physical disturbance, if not death. These manifestations indicate that the animal has been rendered abnormally sensitive to the serum employed, or that it has been supersensitized.

It has been suggested that the same untoward result may occur in man, and that serious disturbance and occasionally death, of patients under treatment for diphtheria and other diseases, in which serums are used, is to be apprehended in certain circumstances as the result of repeated injections of the serum in question, because the serum is not derived from the human subject, but from the horse.

History of Anaphylaxis.

The first observation bearing upon this subject appears to have been made by Majendie⁽¹⁾ in 1839. He found that rabbits which had tolerated two intravenous injections of egg albumin without ill effects, immediately succumbed to a further injection made after a number of days.

Later workers with peripitin, frequently observed that some of their animals died suddenly during the course of their treatment from no apparent cause.

Haricourt and Richet⁽²⁾ in studying the effects of eel serum on dogs, found that they were not able to immunize them against the serum, but that on the contrary, there was an increasing sensibility to it, so that finally the dogs died.

Portier and Richet⁽³⁾ found that if dogs were given a very small dose of a glycerin extract from the tentacles of actinia, and then, in fifteen or twenty days later, were given a second small dose, the animals quickly succumbed. The dose given in each case was so small as to cause no symptoms in the normal animal. They appear to have been the first to use the word "Anaphylaxis" to indicate hypersensitivity to a poison.

Pirquet and Schick⁽⁴⁾ described this syndrome which sometimes follows injections of horse serum in man. They shew that the symptoms of this 'disease' when caused by a second injection may either appear at once, or after a short incubation. They draw attention to the analogy of the tuberculin reaction as a well known instance of hypersusceptibility.

The fact, that guinea-pigs which had been used for the testing of diphtheria antitoxin, frequently died when later given an injection of serum, has been known almost since the discovery of diphtheria antitoxin, but no one seems to have attached any significance to this until 1905. Most of the workers with serum regarded it as an accident pure and simple, or considered that the animal's vital resistance had been lowered by the first treatment. During Ehrlich's visit to this country in 1904, Theobald Smith told him that guinea-pigs which had been used in testing the potency of diphtheria antitoxin became acutely ill or died, if injected subcutaneously several weeks later with several cubic centimeters of normal horse serum. Ehrlich gave this problem to Otto who published the article "Das Theobald Smithsche Phanomen der Serumueberempfindlichkeit" v. Teuthold-Gedenkschrift, I Band 1905. In this work he discusses amongst other

things, the relation of the Theobald Smith phenomenon to the cases of re-injection in man, and cites instances of alarming symptoms following a second injection of antitoxic horse-serum.

Within the past few years much work has been done by many observers in investigating the curious phenomenon of anaphylaxis, the importance of which cannot be overestimated in view of the great increase of serum treatment, and the liability to the occurrence of anaphylaxis in the course of general practice.

Anaphylaxis in the Guinea-pig.

Anaphylaxis can be brought about by the introduction of any strange proteid into the body, substances which in themselves are quite harmless, even when administered in very large doses, and which only appear markedly toxic after a second injection.

No experiment is more simple to perform or more drastic in its results than this one. An animal, say a guinea-pig, is inoculated subcutaneously with a very small dose (1/10 c.c. or less) of a complex albuminous substance such as horse-serum. After an interval, not less than ten days, it is again inoculated intraperitoneally or subdurally, with a second dose. If the second injection be intraperitoneal, the quantity of

serum required to produce symptoms is from 5 to 10 c.c. if it be subdural a much smaller quantity of serum suffices($1/4$ c.c.) and the result is more certain. The first injection sensitises the animal rendering it anaphylactic to the second injection. For a variable time five to ten minutes after the second dose, the animal appears none the worse, but soon it shews signs of nasal irritability and vigorously scratches its nose with its fore paws. About this time it may also cough several times. It then becomes extremely restless and agitated; respiratory embarrassment is marked, its breathing becoming rapid, spasmodic, or irregular. Paralysis first of the hind legs, then of the fore legs sets in; the animal lies helplessly on its side and convulsions are soon followed by death.

The whole process often takes only five minutes, though it may take much longer. A fatal result does not take place in all cases, and even after a marked paralysis of all four limbs, the animal may occasionally recover. Besredka⁽⁵⁾ states that if the second inoculation be made into the peritoneum 25% of the animals die, while if the second injection be subdural, death is the rule. Of fourteen guinea-pigs tested by us, both injections being into the peritoneum, ten died, shewing the typical symptoms of acute anaphylaxis, while four, although extremely ill, recovered. All of these guinea-pigs were inoculated with $1/100$ c.c. of

normal horse-serum, and then after an interval of twenty-one days were again inoculated with 8 c.c. of the same serum. The more complex proteids - egg-white, milk and horse-serum - determine anaphylaxis with great certainty. The less complex proteid materials - peptone - are not so effective, while broken-down products such as leucin and tyrosin are quite ineffective. Rosenau and Anderson⁽⁶⁾ have also been able to produce anaphylaxis with bacterial extracts.

Influence of Time.

To produce this reaction in animals, a certain interval is necessary between the first and second injections. This interval has been found to be not less than ten days, and averages from ten to thirteen days. Otto Rosenau and Anderson⁽⁷⁾ state that when the interval between the ^{two} injections is less than twelve days the animal only reacts very little or not at all, and the curious thing in this connection is, that the animal does not react at all to a new injection of serum, even if the latter were made beyond the period of twelve days from the last injection. In other words, the guinea-pig having received a second dose of serum in the peritoneum before the expiration of the incubation period, becomes vaccinated against anaphylaxis.

Rosenau and Anderson⁽³⁹⁾ admit that this immunity so produced is not very active or lasting, as the

animals when re-injected at a later period again shew symptoms of anaphylaxis. Gay and Southard⁽¹⁴⁾ shew that in the case of a large initial dose of horse-serum, or of multiple doses at short intervals, the animals are not immunized, but are merely rendered refractory, and that this refractory period is simply prolonged in proportion to the amount of serum injected.

Pirquet and Schick⁽⁸⁾ point out that this interval of from ten to thirteen days is practically the same as that required in the human subject to produce the symptoms of serum disease, namely, urticarial eruptions, joint pains, etc. after a first injection of serum. From clinical observations, made in a large number of children treated with anti-diphtheric and anti-scarlatinal sera, they shew that in eight children injected with antitoxic horse-serum ^{and again re-injected} at intervals of from sixteen to forty two days after the first injection they all manifested symptoms of serum disease within twenty four hours. In a further series of sixty children injected at intervals of from six days to seven and a half years after the first injection, they state that when the second injection was given fourteen days to four months after the first injection they obtained an immediate reaction, but when the interval between the first and second injections was over four months

the reaction did not appear for five, six or eight days - what they term the accelerated reaction.

Grünbaum⁽⁹⁾ from observations made with repeated doses of serum, emphasises the importance of accumulation, in the production of the reaction, rather than the importance of the element of time.

The Specificity of the Reaction.

It is only relative. Thus if an animal be inoculated with egg-white, horse-serum, and milk, subsequent symptoms on second inoculation will depend upon the order in which the latter is made. When injected second or third in the series, egg-white alone produces maximal symptoms at all times, horse-serum is diminished in toxicity, and milk almost entirely loses its toxic power.

Besredka⁽¹⁰⁾ states that the toxicity in hypersensitive guinea-pigs is only specific to a certain degree, and that guinea-pigs sensitized with cow's milk do not react to the intra-cerebral injection of human milk, but they react very distinctly to goat's milk which kills them in some minutes.

Rosenau and Anderson⁽¹¹⁾. Guinea-pigs sensitized with horse-serum are very susceptible subsequently to a second injection of horse-serum, but they are only slightly if at all susceptible to a second injection of

serum of other animals such as the rabbit, dog, hog, sheep, chicken or man. This specific nature is more marked when proteid substances of widely different nature are used at the first and second injection; thus if the animal be sensitized with horse-serum, it does not react to a second injection of egg-white, vegetable proteid or milk.

To determine whether the specificity of blood serum was sufficiently absolute to allow of the preparation of therapeutic sera from animals of allied species, a number of guinea-pigs were sensitized by us with 1/100 c.c. of normal horse-serum, and after an interval of from seventeen to twenty one days were re-injected with 8 c.c. of asses-serum. All of the animals shewed the usual symptoms of acute anaphylaxis which did not differ in any way from those animals first sensitized with normal horse-serum, and later tested with the same serum.

Hereditary transmission of Hyper-sensibility.

Rosenau and Anderson⁽¹²⁾ shew that hypersusceptibility to alien serum is capable of being transmitted from the mother to the young, in the case of a guinea-pig, whether the mother is sensitized before or after conception.

They have proved that this transmission is not

effected through the milk of the mother, and also, that the paternal element plays no part in the transmission.

If this be so in the guinea-pig, it is extremely probable that the same hereditary transmission occurs in the human subject, and as supersensitiveness persists for an indefinite period in those persons injected with alien serum, we would then be justified in supposing that all children born after the mother has undergone serum treatment, may be in a state of hypersensibility to that serum. This hypothesis would afford an explanation of those cases of sudden death or serum illness which occasionally occur at the first injection of serum in the treatment of diphtheria and other diseases.

Post-mortem Changes.

Gay and Southard⁽¹³⁾ state that "haemorrhage rather definitely localized is the gross lesion in guinea-pigs dying from anaphylaxis, but that fatty degeneration also occurs. The haemorrhages may be found in one or several organs, but they are most often found in the stomach.

They are minute in general but are rather larger in the lungs and spleen than elsewhere. In the stomach foci of fatty change occur, especially in the gastric epithelium and in the epithelium of neighbouring vessels and haemorrhages are common from rupture of these

degenerated capillaries. Fatty changes were also found in voluntary muscle, heart, and nerve fibres, also in the capillaries of the liver and kidneys, and in the cells of the bulb and spinal cord".

As many of the guinea-pigs dying in this disease, do so in about five minutes and the majority under thirty minutes, it appeared remarkable that definite lesions of fatty degeneration could take place in so short an interval.

To determine the presence or absence of these lesions in the guinea-pigs experimented on in the Liverpool University, I made a series of post-mortem examinations on these animals.

Twelve guinea-pigs, dying within one hour of the second inoculation, were examined; portions of the liver, kidney, spleen, lung and brain, being taken in all cases, heart and muscle fibre in three cases. The naked eye specimens and sections were in every case compared with specimens from control guinea-pigs which were killed by drowning.

The naked eye appearances were simply those of marked congestion of all the organs of the body. The diaphragm appeared more rigid than normal in nine cases, while in the remaining three cases no change could be detected.

Microscopic Examination.

Methods used:- The Müller-Marchi method was used for portions of all the tissues, fixing in Müller's fluid (potassium bichromate 2.5 grms, sodium sulphate 1.0 grms, tap water 100 c.c) for one week, and staining in Marchi fluid (Marchi fluid 2 parts by volume and osmic acid one per cent. solution, one part by volume) for one week. 9

The freezing microtome was also used for portions of all the specimens, hardening being done in 4% formaldehyde for forty-eight hours. The stains used for these sections were Sudan III, which shews up fat as a golden yellow, and Nile-blue sulphate which stains neutral fats red.

Sections were also prepared by the ordinary paraffin method, hardening being done, as before, in 4% formaldehyde.

These sections were stained by Van Gieson's method, and by haematoxylin and eosin. (Van Gieson's stain - $\frac{1}{2}$ per cent. solution of Picric acid, $\frac{1}{2}$ per cent. solution of acid fuchsin, about 20 volumes of the former mixed with 1 volume of the latter. This stains connective tissue pink and muscular tissue yellow).

Liver (Van Gieson) in all cases shewed intense congestion of the intralobular and interlobular blood vessels.

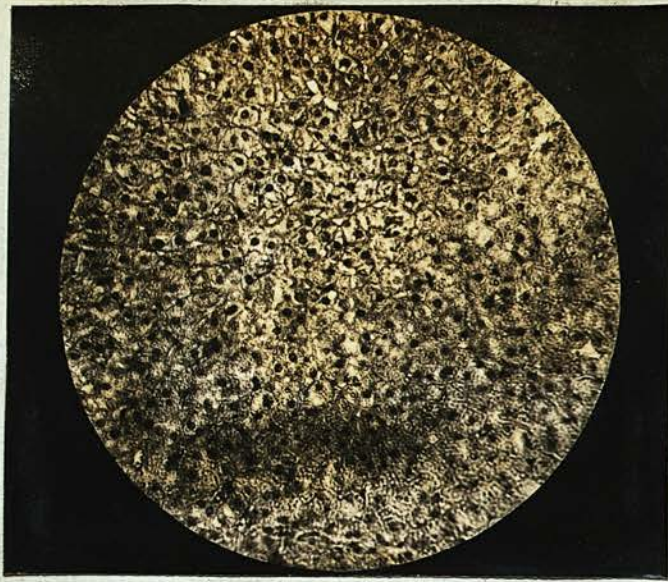
The congestion is distributed in irregular areas the intervening tissue only deviating from the normal structure by exhibiting slight congestion. Interstitial haemorrhages were distinct in two of the specimens but the congestion was so marked in several of the sections that it was impossible to state definitely if the blood has escaped from the vessels into the tissues. The outline of the liver cells in the congested areas is very poorly marked, the cells being distorted and in many cases fragmented and replaced by granular debris, the nuclei being either absent or lying quite free from the cells.

The lobules are not distinct being broken up by intense congestion.

Liver (Marchi) shews no fat either in the liver cells or in the cells of the capillary walls. Liver (Sudan III); complete absence of fat.

The congestion shews well owing to the staining of the blood and vessels.

Liver. (Nile blue sulphate): not one of the sections gave the red staining of fat.



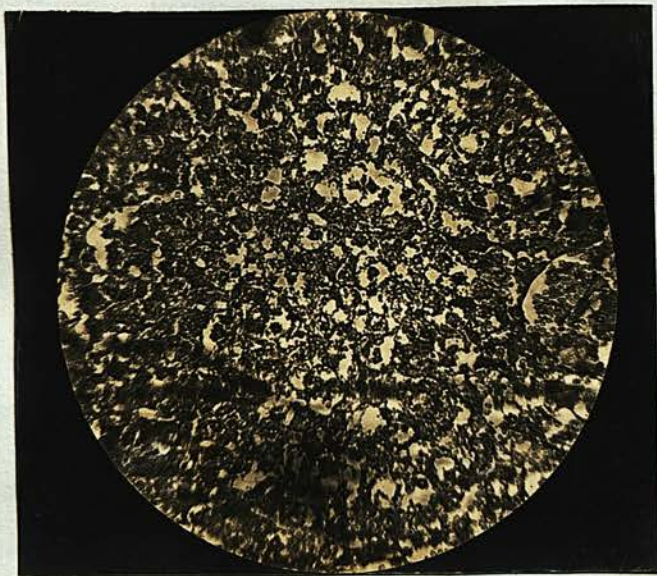
Section I. Normal liver. Stain - Van Gieson. To be contrasted with sections II and IV.



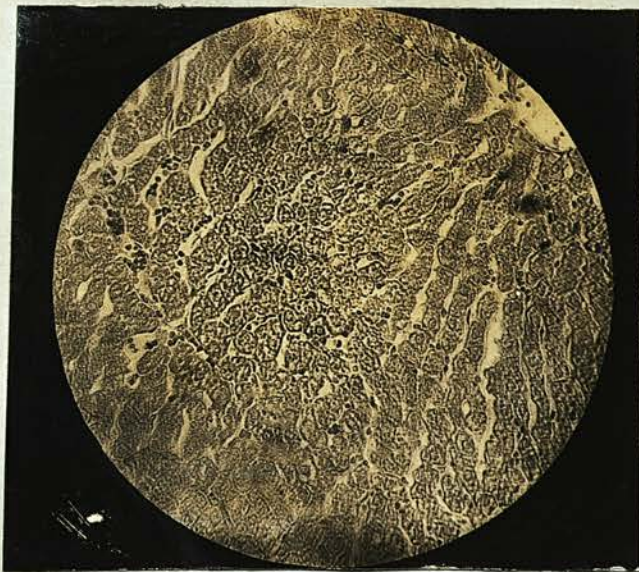
Section II. Liver. Stain - Van Gieson, shewing well marked congestion, interstitial haemorrhages and fragmentation of the cells and lobules with many of the nuclei apparently free from the cells. The cell nuclei stain very irregularly.



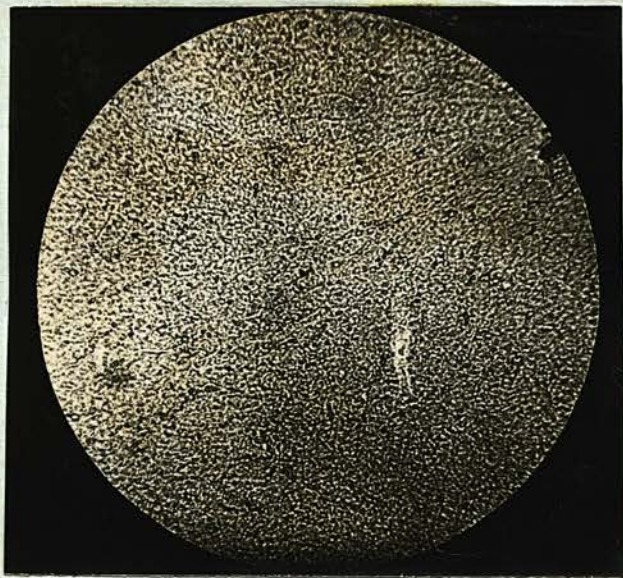
Section III. Liver. Stain - Van Gieson. From same specimen as Section II, shewing the comparative normal appearance of the lobules between the areas of congestion.



Section IV. Stain - Haemotoxylin and Eosin. Shewing the marked vacuolation produced by the congestion with destruction of the liver cells and absence of the outline of the lobules. The granular debris replacing the liver cells shews well.



Section V. Liver. Stain - Marchi. Shews an area of congestion with entire absence of the jet black staining indicative of fat formation.



Section VI. Liver. Stain - Sudan III. Shewing the normal appearance of the liver under low power with this stain.



Section VII. Liver. Stain - Sudan III.

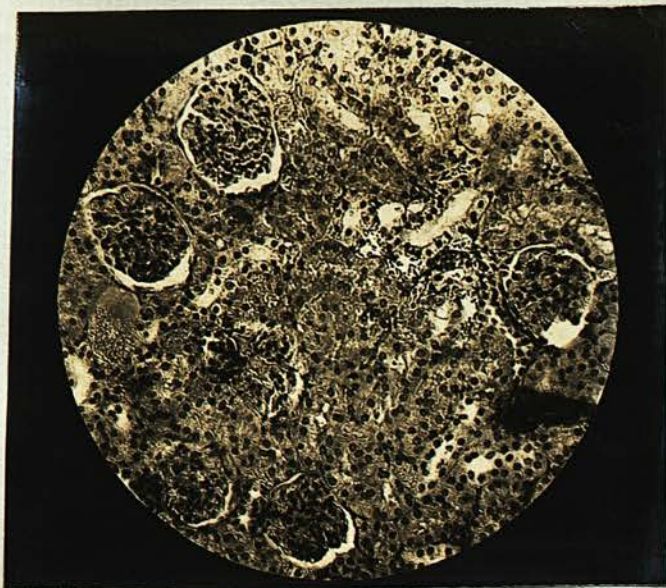
Shewing an area of intense congestion. The lobular outline is better marked owing to the staining of the blood vessels which gives the appearance of an injected specimen. Complete absence of golden yellow colour indicating fat.

Kidney. (Van Gieson). Marked congestion was found in all the specimens especially in the deeper cortex, but also to a lesser degree in the medulla. Four of the sections only shewed interstitial haemorrhages. The cells of the tubules appear to have suffered the most damage. In many parts they are lying free in the tubules and often broken and granular, while in other parts some of the cells have entirely disappeared.

Kidney. (Marchi). No fat could be detected in the capillary walls or in the cells of the tubules. The effects of the congestion is well shewn by the appearance of vacuolation and absence of cell staining.

Kidney. (Sudan III). Shews no fat but some small areas of interstitial haemorrhage were seen.

Kidney. (Nile blue sulphate). Shews no fat in any of the specimens.



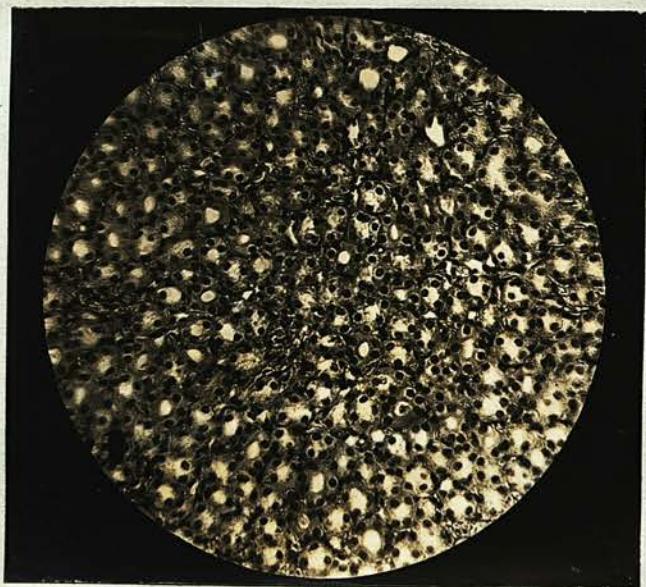
Section VIII. Normal kidney. Stain - Van Gieson.



Section IX. Kidney. Stain - Haemotoxylin and Eosin. Shews an area of congestion. Many of the cells are broken and do not stain well. The nuclei stain deeply and are shewn crowded together in parts by the congestion.



Section X. Kidney. Stain - Van Gieson. More marked congestion. The outline of the tubules is often indistinct owing to the congestion of the tissues around them. The tubules contain many of the fragmented cells and the nuclei in these stain badly.



Section XI. Kidney. Stain - Van Gieson. Small area of normal kidney tissue from same specimen as Section IX.



Section XII. Kidney. Stain - Marchi. Complete absence of black staining indicating fat. The appearance of vacuolation produced by the congestion is seen.



Section XIII. Normal kidney. Stain - Sudan III.



Section XIV. Kidney. Stain - Sudan III.

No fat staining.

Shews dilatation of the vessels with some small areas of interstitial haemorrhage.

Spleen. (Van Gieson). Shews marked congestion with areas of interstitial haemorrhage which gives the appearance of a plexus of large blood sinuses with an island of Malphigian corpuscle in its midst. The true blood spaces contain numerous cells, many of endothelial type, but some resembling the granular splenic cells. Many of these cells appear to have undergone some necrosis and stain faintly, though the nuclei for the most part stain well.

Spleen. (Haemotoxylin and Eosin). The same congestion is apparent. The splenic pulp is broken up and almost lost in parts by numerous haemorrhages.

Spleen. (Marchi). None of the sections contain any fat.

Spleen. (Sudan III). Congestion is not so marked as in the liver and kidney. No fat is visible.

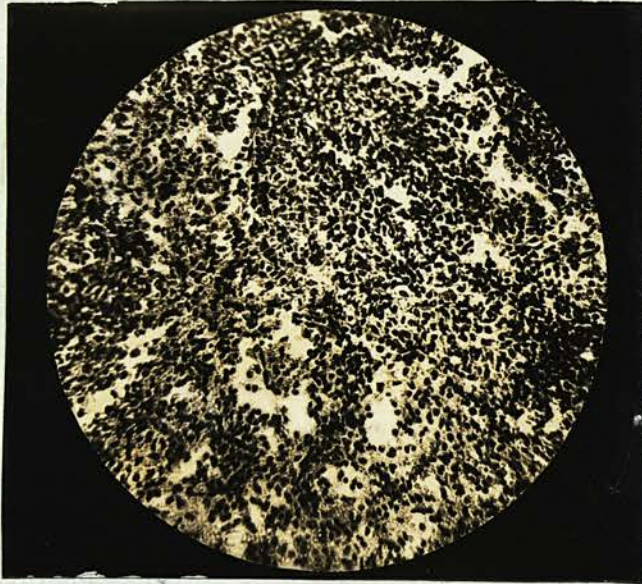
Spleen. (Nile blue supphate). Shews no fat.



Section XV. Normal spleen. Stain - Van Gieson.



Section XVI. Spleen. Stain - Haemotoxylin and Eosin.
Shewing the acute congestion produced and the cells and granular debris lying in the blood spaces.



Section XVII. Spleen. Stain - Van Gieson. An area of slight congestion with more normal appearance of splenic structure.



Section XVIII. Spleen. Stain - Marchi. Complete absence of black staining indicative of fat. Congestion is also distinct.

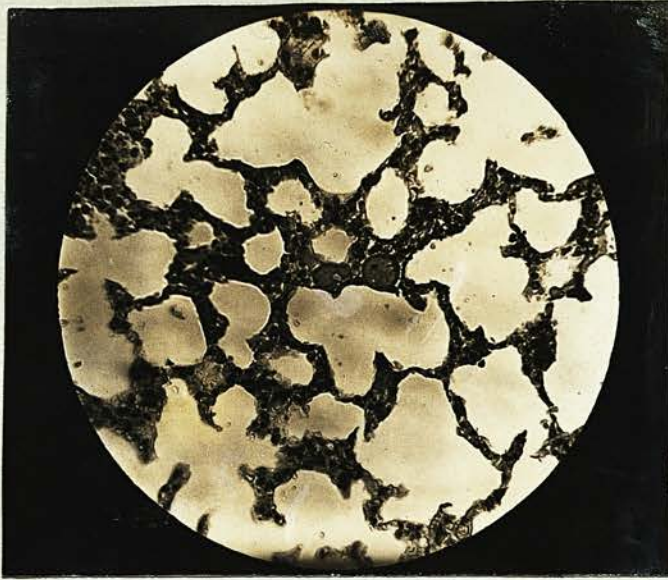
Lungs. (Van Gieson). Areas of intense congestion, more marked especially around the large vessels was present in all cases. The walls of some of the alveoli are so distended with blood that the endothelium lining them is not visible. Many of the alveoli contain blood, and some, the desquamated endothelial cells.

Lungs. (Haemotoxylin and Eosin). The congestion and spots of interstitial haemorrhage are well marked.

Lungs. (Marchi). No fat is visible in the cells lining the alveoli or in the capillary walls.

Lungs. (Sudan III). Congestion and haemorrhage are distinct. No fat visible.

Lungs. (Nile blue sulphate). No fat.



Section XIX. Lung. Stain - Van Gieson.
Shews congestion in the walls of the alveoli, also
some desquamated endothelial cells.

Brain. (Van Gieson). Nothing abnormal could be
found in any of the sections, nor any apparent differ-
ence from those animals killed by drowning.

Brain. (Marchi). No sign of fat staining in any of
the sections.

Brain. (Sudan III). No fat.

Brain. (Nile blue sulphate). No fat.



Section XX. Normal brain. Stain - Marchi.



Section XXI. Brain. Stain - Marchi.
No black staining indicating fatty degeneration.



Section XXII. Brain. Stain - Haemotoxylin and Eosin.
Nothing abnormal was noted in any of the specimens.

Voluntary and involuntary muscle fibres differed in no way from specimens taken from the animals killed by drowning. In none of the sections was any fatty degeneration found.

From the results of these examinations it would appear that the only departure from the normal is to be found in the marked congestion of the various organs, with small interstitial haemorrhages in some cases; and that there is no fatty change either in the cells of the blood-vessels or in the cells of the organs or tissues. The importance of the post-mortem examinations cannot be overlooked as they shew there are not any gross cell lesions, at least demonstrable by definite cell changes, and whatever the cause of the untoward results of a second injection of serum, it can hardly be explained by the microscopical changes found in the organs or tissues of the body.

Theories of Anaphylaxis.

Several theories have been put forward explaining this reaction, the more generally accepted, however, are those of Besredka, and Gay and Southard.

It has been proved, that serum, when heated to 100°C. before the first inoculation, is still able to so affect the animal that on the second inoculation anaphylaxis occurs. There is therefore in serum a thermostabile substance which prepares the animal for the second dose. This substance has been called Anaphylactin by the Americans; Sensibilisinogene by Besredka. On the other hand, if the serum be heated to 100°C. before the second inoculation the symptoms of anaphylaxis do not appear. The substance, therefore, which produces the final crash is thermolabile, and is spoken of by the American school as the toxic portion; by Besredka as antisensibilisin. We have, then, two substances in serum - firstly, a thermostabile substance which prepares or sensitises the animal; secondly, a thermolabile substance which produces the symptoms. Upon this experimental evidence the theories of Besredka and that of Gay and Southard have been propounded.

Besredka⁽¹³⁾. One can admit the presence of two substances in all normal serum, one of which has

the character of antigene, and the other that of anti-lysine; the first we call Sensibilisinogene, and the second Antisensibilisin. The Sensibilisinogene has the power of giving rise in the guinea-pig, twelve days after the injection of serum, by acting on its cells, to an antibody Sensibilisin which fixes itself on the cells of the nervous system and which unites as fast as formed with the Antisensibilisin contained in the inoculated serum. So long as there is any Antisensibilisin left, this combination proceeds, and explains the refractory period. But the amount of Antisensibilisin is soon used up, being fixed to the Sensibilisin already formed.

The Sensibilisinogene however, goes on acting on the cells and producing Sensibilisin for which there is no antibody and so it tends to accumulate in the body being either fixed to the cells of the nervous system or free in the blood. Now if a second injection of serum be given, the Antisensibilisin contained in the serum meets the Sensibilisin in relatively large amounts, and combines with it, with explosive violence causing a sudden de-sensitization which manifests itself either by serious anaphylactic phenomena or death.

Gay and Southard(14 & 15). There is a substance anaphylactin in horse-serum not absorbed by the tissues of the guinea-pig, not neutralized, and very slowly eliminated from the body.

In the injected animal, the anaphylactin remains irritating the body cells, and increasing their avidity for the other elements of horse-serum which are quickly eliminated or destroyed. No antibodies are produced by anaphylactin. After two weeks' stimulation by this substance, the body cells, if suddenly presented with a large amount of horse-serum, are overwhelmed in the exercise of their increased assimilating functions, and equilibrium is so disturbed that local or general death may occur. In the case of a large initial dose of horse-serum, or of multiple doses, the incubation or refractory period is prolonged in proportion to the amount of serum injected, because the cell avidity cannot begin to increase until the assimilable substances of the serum are largely eliminated, and the normal assimilating function of the cell is unsatisfied. When this increased assimilating power has been satisfied the further addition of serum produces no effect. They assert the change is essentially within the cells, and that it is evidenced by the production of fatty degeneration; this, however, has not been confirmed.

Vaughan⁽¹⁶⁾ advances the theory that on the first injection of a strange proteid, it is broken up into components, one of which is toxic, but as the breaking-up takes place slowly, the animal is not poisoned. The cells from this lesson, however, learn how to break up the complex molecule, so that, at the second injection, it is violently rent asunder, and quickly liberates large quantities of the toxic principle of the complex molecule.

Currie⁽¹⁷⁾ suggests that the first injection of serum results after an interval in the formation of an antibody. When the second injection is given at least ten days later, the antibody producing substance of the second injection of serum, and the antibody produced by the first injection come together without delay. The union is rapid, the whole charge of the poisonous substance is quickly set free and the toxic symptoms are sudden and severe.

Grünbaum⁽¹⁸⁾. "There are two factors which have not received much attention in the explanation of the phenomenon of anaphylaxis - the constitution of the serum and the condition of the patient.

Rosenau and Anderson believe that the antitoxin in anti-diphtheric serum plays no part, but they also find that the presence of some toxin appears to

increase the supersensitiveness. Consequently, while the reaction depends mainly on a substance peculiar to the alien serum, it may be assisted by the presence of toxin, perhaps neutralized in the serum as injected, but afterwards dissociated in the body. In anti-tuberculous serum it is more likely that the antitoxin is minimal in amount, and the curative action is largely due to a minute quantity of Tuberculin. But the presence of the Tuberculin may help to induce a supersensitization, and by continuous injection produce a deep negative phase of diminished resistance. The alien serum itself also acts like a vaccine, so that each succeeding administration taking place during a negative phase, leads up to the final catastrophe. Probably personal idiosyncrasy is the chief factor and this can be determined only by trial".

Kraus and Volk⁽¹⁹⁾ support the theory of Besredka based on the presence in the serum of two distinct properties, Sensibilisinogene and Anti-sensibilisine.

Biedl and Kraus⁽²⁰⁾ shew that the re-injection intravenously of serum is followed by a lowering of arterial pressure which is in itself sufficient to explain all the symptoms of anaphylactic accidents, such as excitation following depression, defaecation,

anuria, etc. The arterial depression is due to paralysis of the peripheral vasomotor apparatus. They prove that the peripheral vasomotor paralysis is real, by the subsequent use of barium chloride again raising the arterial pressure. With this fall of arterial pressure, they find there is a diminution or even an absence of the ^{coagulability} coagulability of the blood. They state that anaphylactic intoxication is provoked by a poison which is identical, physiologically, with the peptone of Witte.

Charles Richet⁽²¹⁾. In 1902 he found marked lowering of the arterial pressure in anaphylaxis. He questions if the vasodilation of Biedl and Kraus is a primitive peripheral phenomenon, and not a secondary phenomenon due to a central innervation trouble, as barium chloride can act on the central as on the peripheral nerves.

Otto and Friedmann⁽²²⁾ have been able to produce passive sensitization by injecting the serum of supersensitized animals. Therefore the active substance is not attached to the cells in the vital organs, but is free in the blood of the animals, and therefore there is reason to think that the reactions under discussion are related less to sessile receptors of vital cells than to free receptors.

Nicolle and Abt⁽²³⁾ state that albumino-coagulin or precipitin, and albumino-lysin play a part in serum reactions, but that both are primary. Though precipitation is not effected in the living body, it is held, there takes place in the body a coagulation or condensation of the foreign albumin, by which its potential activity is lessened. Albumino-lysin again becomes active after albumino-coagulin, it dissolves the compound of extraneous serum and albumino-coagulin, and liberates a poison of the nature of endotoxin against which the body has no protection (Wolff 1904). Hence the action of albumino-lysin is injurious.

Nicolle and Pozerski⁽²⁴⁾, hold that large doses of serum evoke albumino-coagulin, while small doses favour albumino-lysin. Large doses should therefore tend to mild serum reactions, and small doses to severe reactions.

Von Pirquet and Schick⁽²⁵⁾ assert that in man large doses of serum are more active than small doses, in predisposing to abnormal reactions. They shew that there is no definite relationship between precipitin formation and the serum reaction, so that there are therefore points in the theory that two primary antibodies determine serum reactions, which are difficult to reconcile with the facts under notice.

Anaphylaxis in Man.

In the treatment of diphtheria with anti-diphtheric serum, patients having received a single injection, or a number of injections at short intervals, frequently exhibit signs and symptoms of what has been called the normal serum reaction. This occurs after an interval of some days which varies slightly in different cases, but rarely occurs before the tenth day, and usually before the fifteenth day. It consists mainly of a skin rash, which is urticarial and itchy in character, and is usually associated with joint pains. There is often slight fever (100° - 101°), and in some albuminuria is present. These symptoms usually completely disappear in from two to four days.

The abnormal serum reaction, on the other hand, is of a much more serious nature. It has given rise to alarming symptoms in many cases, and in some has been considered to be the direct cause of death. We have seen how, in the guinea-pig and other animals, the second injection of serum, after a definite incubation period, acts as a deadly poison and usually kills the animal in a few minutes.

It does not appear unreasonable to suppose that the same result may occur in man, and indeed Otto and the Americans⁽²⁶⁾ see a close connection between

anaphylaxis in the guinea-pig, and the symptoms one sees in man after a second serum administration. Pirquet and Schick⁽²⁷⁾ divide this abnormal reaction into "immediate" and "accelerated" reactions. The "immediate" reaction is the occurrence of local oedema, urticaria, erythema, and constitutional disturbances apparent within 24 hours of the second injection. The "accelerated" reaction is a train of symptoms differing from the results of a single injection, in their earlier onset, briefer duration, and frequently severer course. They state, that in eight children injected with horse-serum and again re-injected at intervals of from sixteen to forty two days after the first injection, they all shewed symptoms of serum disease within forty eight hours. In a further sixty children injected with antitoxic horse-serum at intervals of from six days to seven and a half years, between the first and second injections, when the second injection was given fourteen days to four months after the first injection, they regularly obtained the immediate reaction, but when the interval between the two injections was over four months, they only got the accelerated reaction - fever, urticaria and other symptoms of serum disease, - appearing on the fifth, sixth, seventh or eighth day. After six months interval, the immediate reaction is rare, and

and they hold, in apposition to Otto, Rosenau and Anderson, that a large primary dose of serum favours the immediate reaction.

A. Grünbaum⁽²⁸⁾ gives five cases in one of which death occurred, and four, alarming symptoms took place during the administration of Marmorek's anti-tuberculous serum.

The serum was given on successive or alternate days for several doses, followed by an interval of several days before the next series of doses, the bad effects always occurred in the course and not at the commencement of a series. The patient who died received doses of 5 c.c. to 9 c.c. on successive or alternate days from January 20th to March 8th, with intervals of nine, six and eight days respectively. Almost immediately after the last injection, he complained of feeling queer, became cyanosed, vomited, lost consciousness, and died within five minutes.

A second patient after eighteen injections of 103 c.c. given much similarly, began to feel faint, felt blood rush to the head, had a choking sensation and thought she was becoming blind. Objectively she became suffused, a deep bluish-red appearing on the forearms, thighs and face. Later she vomited, pulse became 120 and remained so for twenty four hours. She complained of pain in the front and back

of chest for one day.

A third case after sixteen injections of 76 c.c given similarly, two minutes after the last injection, felt a tightness at the throat, became unable to see, and lost consciousness. She lay flat in bed, waving her arms about, with eyes turned up and face twitching. This condition lasted about one minute. With returning consciousness she complained of pain in the head, tightness and pain on the right side of chest, back and front. Her pulse remained quick for twenty four hours.

Currie⁽²⁹⁾ states that Otto(p.18) and Pirquet and Schick (1905, p.98) each had a case which came near death, and Rolleston (1905,p.664) refers to grave symptoms which may ensue within a few hours of injection of serum in case of a relapse or of a second attack of diphtheria. He continues - from observations cited, it is apparent that there occur in the human subject after two or more injections of horse-serum symptoms which though much less severe are comparable with the phenomena of supersensitization induced in the guinea-pig by similar treatment with serum of animals of a different species. The interval of time is the primary factor in determining these abnormal reactions.

Later⁽³⁰⁾ of fifty cases treated for cerebro-spinal fever with serum, twenty nine had rashes, fifteen of these shewing the normal reaction only, seven shewing both normal and abnormal, while seven had the abnormal reaction. Of twenty six cases re-injected after the ten day interval, fourteen had abnormal reactions, while of twenty four cases re-injected within the ten day interval, none had abnormal reactions. He concludes that the primary conditions for an abnormal reaction to appear, is the lapse of a certain interval of time, approximately ten days, between the two injections, but that a further lapse of time, beyond this interval has no additional influence in inducing the phenomenon. Rosenau and Anderson⁽³¹⁾ have shewn that anaphylaxis can be produced in the guinea-pig by means of bacterial extracts.

A case of vaccine anaphylaxis which took place while under my care seems to be of interest in this relation.

The patient, a hospital nurse, aged 58, was being treated with diplococcus catarrhalis vaccine. Injections were begun on July 14th, 1909, and repeated three times at intervals of eight, six, and five days respectively, in doses varying from 117,000 to 500,000 diplococci. The local reaction was never

excessive except after the initial puncture. A slight feeling of sickness was noticed after the third and fourth inoculations. The temperature rose to 101° after each injection, but never exceeded this; the pulse rate was not disturbed.

The temperature did not settle until five days after the fourth injection, so that further inoculations were postponed for a few days longer.

On the twelfth day from the last injection (Aug. 13th) a dose of 294,000 diplococci was given in the arm as before. The following morning (Aug. 14th) the patient complained of intense headache, frontal and occipital, accompanied by a feeling of sickness. Vomiting began about mid-day and continued at short intervals until early in the morning of the 15th Aug. everything including sips of water being at once returned. This was associated with cramp-like pain in the region of the stomach radiating to the lower part of the abdomen, intense headache, cold hands and feet. Later the same day, the pains spread to the groins, legs and calves, in the latter especially the pain was intense, while the abdominal pains became much less. The temperature which had been normal up to the morning of the 15th Aug. rose rapidly to 102.2 at mid-day, returning to normal at 10 p.m. the

same night. The same evening patient had several attacks of shortness of breath, and had to sit up in bed panting, this was accompanied by dizziness and things floating before the eyes. The next morning, Aug. 16th, patient was bathed in profuse perspiration, which had continued through the night. All the pains had gone except the intense headache which still persisted. There was complete absence of the desire for food, the stomach, as explained by the patient, feeling quite full. Two days later the patient felt quite well, the headache having disappeared, and the appetite returned. The pulse always remained good and never exceeded 98.

Prevention of Anaphylaxis.

Besredka⁽³²⁾ finds immunity can be conferred on guinea-pigs by injecting 1/4 c.c. of serum under the dura before they are rendered completely anaphylactic, that is, before the expiration of twelve days from the first injection. Also in guinea-pigs already in the anaphylactic state, by introducing a very weak dose of serum 1/40 c.c. to 1/400 c.c. into the peritoneum, not only does no morbid symptom appear, but further, the animals are rendered completely immune. This immunity lasts at least three months. He⁽³³⁾ tried

to vaccinate sensitized guinea-pigs against anaphylaxis by the digestive methods. The rectal method only has given positive results. Guinea-pigs which received after lavage 15 c.c. of serum into the rectum, resisted in the majority of cases, the fatal dose of serum afterwards injected into the brain.

He⁽³⁴⁾ states that serum is diminished in toxicity by age. It is very toxic the day it is obtained, towards the eleventh day its toxicity is diminished by half and continues diminishing for two months, after which it remains stationary for several months.

Moderate and prolonged heating of the serum, he says, markedly diminishes its toxic power while the antibody remains intact. Thus he advises heating the serum to 59°C. or 60°C. from one to seven hours, and he finds this diminishes its toxicity from four to five times. In view of this, he states, the reason the French sera are much less toxic than others, is because they are subjected to heat of 55°C. before being injected. Further⁽³⁵⁾ he found that anaphylaxis could be prevented by means of narcotics. If the second injection of serum were given while the animal was asleep, it wakened later not having presented any symptom of anaphylaxis.

He states⁽³⁷⁾ that all serum before being used for man should have its toxicity first tested on the guinea-pig, and that it should be avoided if the dose of 1/20 c.c. made into the brain of the sensitized animal causes a fatal termination.

Otto⁽³⁸⁾ shewed that immunity to the poisonous action of serum injection may be acquired by repeated injections of large amounts of serum at short intervals

Rosenau and Anderson⁽³⁹⁾ state, this immunity is not quite so lasting and definite as many instances of active immunity produced against bacterial infections. Guinea-pigs which have received a number of prior injections of horse-serum may again shew symptoms when re-injected but the symptoms in such cases are mild, and death has never occurred in an immunized guinea-pig as a result of subsequent injections of horse-serum.

They believe⁽⁴⁰⁾ that it is advantageous to use the precipitated sera, as the same number of units can be given in half the bulk, and that the untoward effects of serum administration depend to some extent upon the volume of serum administered.

Netter⁽⁴¹⁾ has shewn that when one gramme of calcium chloride is given on the day of injection, and on the two following days, the number of children

showing the eruption following the injection of serum is greatly diminished.

Rosenau and Anderson⁽⁴²⁾ thought calcium chloride might have some influence on the phenomenon produced in guinea-pigs by two injections of serum, but we find, however, it may modify the occurrence of rashes in children following a single injection of serum, but it does not influence to any marked extent the toxic effect in guinea-pigs of a second injection of serum given fourteen days after the first injection.

Grünbaum⁽⁴³⁾. All cases of repeated injections of alien serum should be done with great care, and should be intermitted for a period as long as compatible with the safety of the patient as soon as the first sign of serum disease appears. In all cases where rectal administration is efficient, it should be preferred to subcutaneous injection.

McClintock and King⁽⁴⁴⁾ conclude that the sensitizing action of horse-serum given by the mouth is not nearly so great as when given subcutaneously or intraperitoneally.

Goodall⁽⁴⁵⁾ states that an abnormal reaction is most likely to occur after serum at a second attack, when the patient has had a large volume of serum followed by a reaction at the first attack.

Conclusions. — .

It is well known, that repeated injections of horse-serum into man, is not an infrequent occurrence, and that it is not rare for persons to have several attacks of diphtheria at long intervals, and to be treated each time with anti-diphtheric serum.

If then, we admit that such an event as anaphylaxis may occur in man, the question at once arises, is the use of sera at all justifiable in the treatment of disease?

The answer to this can hardly be other than in the affirmative; for when, we consider the case death-rate of diphtheria now, and compare it with the case death-rate of pre-serum days, it is obvious that whatever dangers there may be in the use of serum, they are infinitely less than those of the disease. This is more especially appreciated when we see recorded the successful treatment of such a disease as tetanus, which was universally fatal in pre-serum days.

Admitting then that the use of serum is justifiable, any precautionary measures that we are aware of, which are likely to minimise the risk of anaphylaxis, should be adopted. These can be divided into two groups, namely, those to be taken by the makers of the serum, and those to be taken by the physician.

We know that the portion of the serum which produces the toxic symptoms is thermolabile, at 100°C.

it is destroyed; at 76°C. it is almost destroyed. But these temperatures are so high that they would destroy or greatly diminish the antitoxic properties of the serum. On the other hand, heating the serum to 56°C. for one hour, on from four to seven successive days very greatly diminishes its toxicity, and does not greatly interfere with the antitoxic power of the serum.

Again, we have seen that the toxicity diminishes with the age of the serum up to two months.

It is therefore the duty of the maker to see that all sera are satisfactorily heated, and kept for a period of not less than two months before disposing of them for therapeutic purposes. Precipitated sera should also be prepared, as by this means the bulk of serum administered is greatly reduced, and with it the risk of anaphylaxis.

The physician must seriously consider whether the use of prophylactic doses of serum is advisable in those suffering from respiratory disease, especially asthma, since it is in these diseases that anaphylaxis is most likely to appear.

When disease is present, the serum should be administered where possible in one large dose rather than several small ones, for two reasons - first, that

an excess of antibody may be introduced as rapidly as possible, to the great benefit of the patient, and second, to reduce to a minimum the necessity for repetition of the dose. Where repetition is necessary or is likely to be necessary, the second inoculation should be given before the lapse of ten days, to endeavour to keep up the refractory period.

The family history, especially of children about to be inoculated, should be enquired into, to find out if possible whether the mother, at any time previous to the birth of the child in question, had undergone antitoxic treatment, as it has been shewn that hypersensibility to alien serum can be conveyed from the mother to the child in the case of guinea-pigs. If the mother had been treated by serum inoculations, the first administration to the child should be as small as possible, the dose to be repeated or increased as required after a short interval if nothing abnormal appears.

Where practicable, the serum should be administered by mouth or by rectum, rather than by subcutaneous inoculation, as this appears to minimise the toxic action of the serum.

If the patient has exhibited a serum reaction at the primary inoculation, a second administration

of serum after the ten day interval should be undertaken with great care in regard to a small initial dose.

If after the administration of serum, the symptoms of anaphylaxis appear, the physician may remember that Besredka shewed that animals narcotised with ether before, during, and for half an hour after the second dose of serum, were saved alive and refractory while the controls all died. So that it is possible in such cases the free administration of ether might save the life of the patient.

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